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ABSTRACT

Optical Tweezing is a non-invasive technique that can enable a variety of single cell experiments or cell-cell communication experiments. To date, optical tweezers tend to be based on a high numerical aperture microscope objective to deliver the tweezing light and image the sample, which introduces restrictions in terms of flexibility. A single optical fibre-based probe able to manipulate microparticles independently from the imaging system is demonstrated. The working principle of the probe is based upon two crossed beams that can be used to trap a microparticle in the area where the two beams overlap. The two deflected beams are produced by incorporating fibre-end facet mirrors onto a multicore fibre using a Focused Ion Beam fabrication technique. The light from the two cores overlaps close to the end of the fibre and has been demonstrated to be capable of trapping particles in the area where the beams intersect. By using a multicore fibre instead of separate fibres glued together results in simplified probe manufacture and alignment and offers a smaller probe that is suitable for use in a wider range of applications, including on-chip manipulation.

Keywords: optical manipulation, optical fibres, multicore fibre, optical tweezing, Focused Ion Beam, microfabrication

1. INTRODUCTION

Optical Tweezing is a technique that uses the momentum of light to manipulate microscale objects. The concept, first demonstrated by Ashkin *et al* [1] is to trap the particle by focusing the light of the laser beam through a high Numerical Aperture (NA) objective. Throughout the last 30 years, this technique has seen widespread applications in physics and in biology. The applications include confinement and arrangement of particles and cells, for example cell-sorting, and measurement of picoNewton forces. A more detailed study of particles or cells can be achieved by combining optical tweezers with microscopy such as Raman and Super-resolution microscopy. To date, optical tweezers tend to be based on a high NA microscope objective to deliver the tweezing light and image the sample. This introduces restrictions upon the optical microscope.

The development of a fibre-based optical tweezing system can overcome these restrictions and increase the flexibility. The first attempt for a fibre-based tool was the two-opposed fibres pattern where the optical forces could be counter-balanced along an optical axis [2]. However, this system demands a strict alignment of the two fibres and does not contribute to the improvement of the flexibility issue. Four years later , Taguchi *et al* [3] suggested a spherically tapered fibre which could optically trap and manipulate particles, but only in two dimensions. Three dimensional trapping based on a single fibre was first demonstrated using a pattern which was chemically etched into a tip-end fibre [4]. However, the trapping point was very close to the fibre end and that made the manipulation process difficult. A significant aim for single-probe optical tweezers came from Liberale *et al* [5], with a proposal for four separate, machined optical fibres glued together within a glass capillary that could demonstrate 3 dimensional trapping of a polystyrene microbead. Although the geometry that allows the beam to undergo Total Internal Reflection (TIR) results in 3D trapping, the probe demands high precision in terms of manufacturing and issues such as the heating up of the glue during a trapping experiment can occur.

In this work, the development of a flexible optical fibre-based probe able to manipulate cells under any type of microscope, such as Raman, microscope has been demonstrated. Mirror-shaped facets have been fabricated at the end of the core region of a four-core fibre using Focused Ion Beam technology (FIB) to achieve a TIR geometry that focuses the light close to the end of the probe. Here, we demonstrate a machined multicore-fibre-based probe that can focus the light close to the

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end of the fibre, with a NA equivalent of a microscope objective equal to 1.103. The effectiveness of the pattern has been tested by trapping yeast cells of 7 μm diameter.

2. DESIGN, FABRICATION AND SETUP

2.1 Multicore-fibre based probe design

As depicted in Figure 1b, mirror-shaped facets have been fabricated at the end-face of a multicore fibre with outer diameter 150 μm and distance between the two diagonal cores 65 μm. The mirrors have been machined so that the light guided through each core can experience TIR at the interface of the medium and the fibre (Fig.1a). The mirror is a pattern of 18 μm x 18 μm square. In our system where the fibre is made from pure silica core and doped silica cladding and is immersed in water, the critical angle is equal to 64.79°, thus the angle of the fabricated mirrors will be slightly higher than this value. The beam is totally internally reflected at the fibre-media interface and then exits the fibre by refraction at a different position at the fibre media interface. Finally, when both of the cores guide light, the two beams cross close to the end of the fibre, similar to the focusing of the outer rays in a high NA objective.

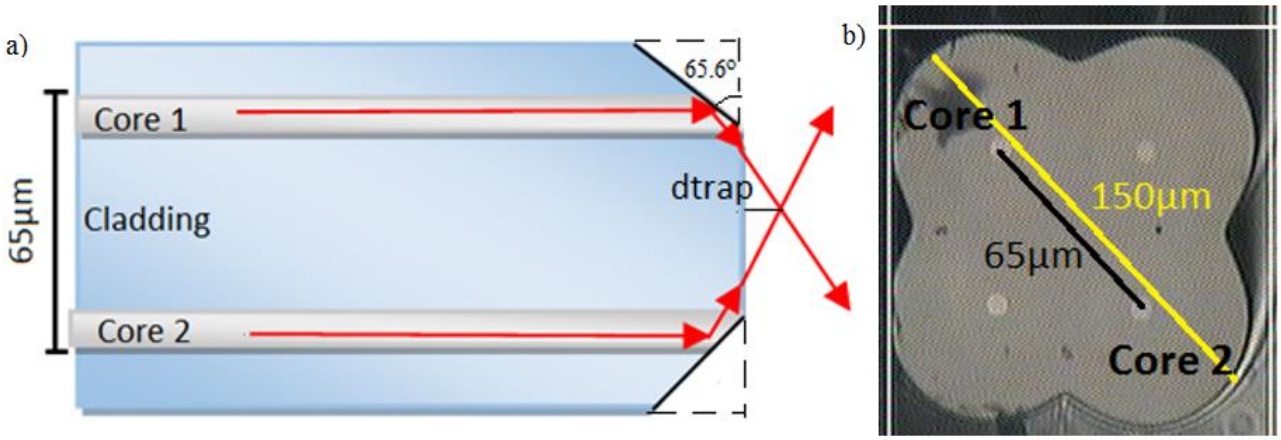

Figure 1: a) Geometry of the light coming through the core, in a machined multicore fibre b) Multicore fibre cross section captured with an optical microscope.

The Numerical Aperture of the probe is measured to be 1.103, for a mirror angle of 65.6°. The distance between the two cores is 65 μm, so the trapping distance away from the fibre end is estimated to be 21.84μm.

2.2 Focused ion beam fabrication of multicore-fibre based probe

To micromachine the facets at the end of the fibre, FIB technology was used. The FIB system (FEI Quanta 3D FEG) consists of a Gallium ion beam gun and a Scanning Electron Microscope (SEM) held together at an angle of 52° (Fig.2a) The ion beam reaches and hits the surface of the sample and sputters a small amount of material, with a beam spot size of 1 Angstrom, leaving the surface with secondary electrons, secondary ions or neutral atoms. The electrons and the ions are collected by a detector to form the image. The surface of the fibre was first coated with ~200 nm thick gold layer through vapour deposition (Edwards Auto 306), in order to prevent electrostatic charging. The ion beam scanned and removed material from a square-shape area of 18 μm x 18 μm at the surface of the two diagonal cores. The depth of the mirror slots were 40 μm. After the fabrication, the gold layer of the area between the two mirrors was removed by using the same parameters for the FIB to avoid absorption during the optical trapping experiment.
By using a low beam current of 7 nA for the whole procedure, we could achieve imaging resolution of tens of nanometers, resulting in high fabrication precision.

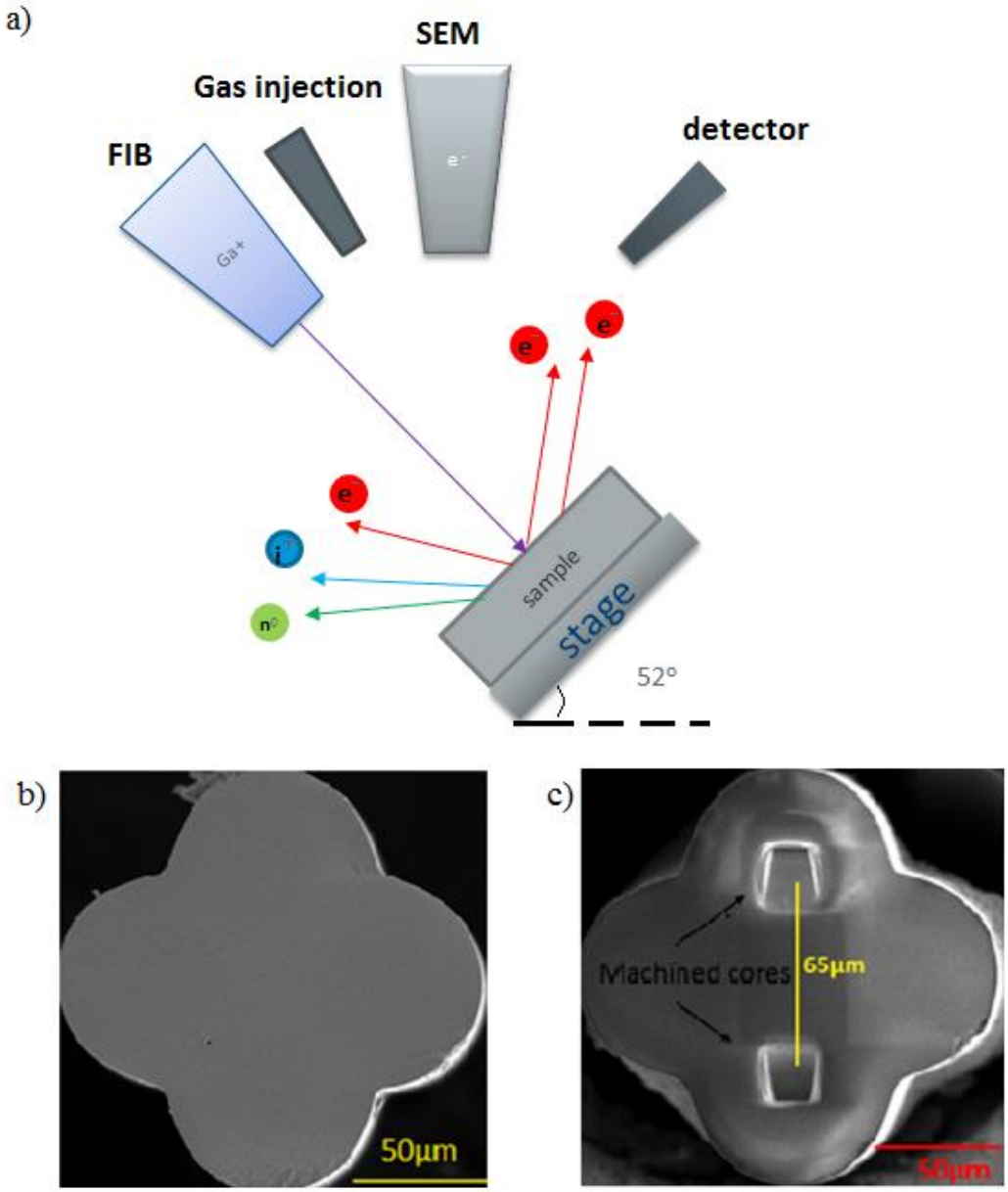

Figure 2: a) Focused ion Beam set up and working principle b) Scanning electron micrograph (SEM) image of the fibre surface cross-section before after the mirror fabrication using the FIB technology. Here, the cores are not visible because of the gold coating of the surface and c) SEM image of the fibre surface cross-section after the mirror fabrication.

2.3Characterization of the multicore-fibre based probe

2.3.1 Fan out and splicing of the optical probe

Once the fabrication is finished, the optical fibre is connected to the laser sources. Three laser sources (Thorlabs, CLD1015) have been connected to the multicore fibre, by splicing the multicore fibre to a fan-out device. This fan-out includes three single core fibres thermally spliced into a four-core fibre. The splicing of the optical probe into the fan out has been done using a thermal splicer (Fujikura ARCmaster FSM-100P). The fan-out device was connected to the

multicore fibre in an orientation that allowed for light to propagate through the two machined cores, in addition to one of the non-machined cores, which acted as a reference.

2.3.2 Set up of trapping experiment- Microchannels fabrication

The trapping experiment demands a set up that includes: three diode lasers of 975nm wavelength (Thorlabs, CLD1015), the fan-out that connects the multicore to the single core-fibre based laser diodes, a microscope slide to hold the fibre stable inside the sample area, a micro-translation stage to mount the sample and the fibre, an external illumination source to illuminate the sample from below, a microscope objective and a CCD camera to image the process, as in Fig.3

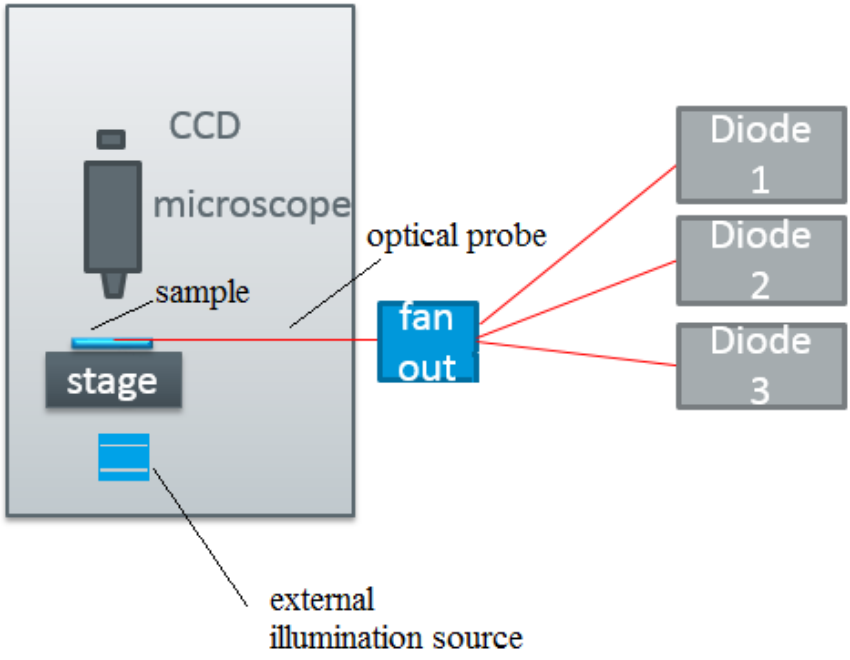

Figure 3: Experimental set up used for the optical trapping experiment.

To prepare a substrate to be able to hold the fibre stable and aligned inside the sample, microchannels at the surface of a borosilicate microscope slide were fabricated using a picosecond laser (TRUMPF, TruMicro 5250-3C) as shown in Fig.4.

The picosecond laser wavelength was 515nm and the parameters used for the fabrication were 40 kHz PFO, number of repetitions N=25, speed 120mm/sec and power P=80%. The above parameters of fabrication have been chosen after experimental testing in trial arrays in order to achieve the desired depth of the microchannels and the smallest damage of the surrounding material. The central square-shaped area in the middle of the microscope slide is the sample area which is deeper than the microchannels.

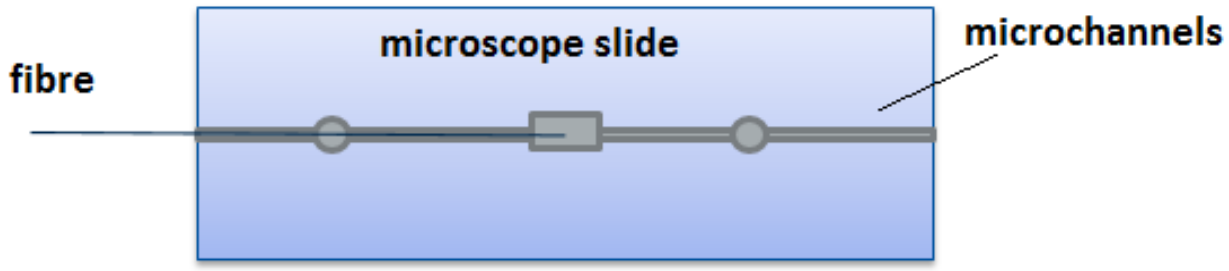

Figure 4: Machined microchannels at the surface of a borosilicate microscope slide in order to prepare a substrate able to hold the optical probe stable and aligned.

2.3.3 Beam profile in water

To evaluate the mirror fabrication, a beam profile was carried out. The beam profile took place inside water in order to simulate the propagation of the beam as it appears during a trapping experiment. The set up for this process included a holder for the fibre, a tank filled with water, a micro-translation stage and a micro-rotator, a CCD camera connected to an objective to image the beam spot. The CCD camera faces the optical fibre so that it captures the spot of the beam coming out of the core. The position where the beam spot of the un-etched circular core is circular, was used as a reference point until the beam spot of the fabricated core appears circular as well. This angle as defined by the rotator gives the experimental fabrication angle that was achieved using the FIB machining. The second step of characterization was the estimation of the beam divergence. For this purpose, we took several spot sizes at several distances of the fibre away from the camera. Having these pairs of measurements, we finally estimated the beam divergence for the two cores to be 4.43^{o} for the first core and 6.52^{o} for the second.

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1Optical trapping and manipulation experiment

To test the capability of this fibre-based probe to optically trap and manipulate microparticles, we demonstrated an experiment using yeast cells (*S. cerevisiae*) of 7 μm diameter. The use of two separate laser sources for each core allows for the combination of powers to be varied to find the optimal combination for each experiment.
The optical power that was transferred to the medium through each core was not higher than 3mW from each core and the trapping distance of the yeast cells fluctuated between 21 and 23 μm away from the fibre end. Single yeast cells could be trapped in three dimensions. The cell could be manipulated in several ways. Firstly, the power of the two beams could be varied, thus the cell could be manipulated small distances (up to 8μm) within the overlap area. Secondly, the fibre itself could be moved within the sample in x, y and z using a fibre micro-translation stage. Thirdly, the sample could be moved with respect to the fibre by translating the sample stage.

Yeast cell manipulation by the fibre probe can be seen in Fig.5.

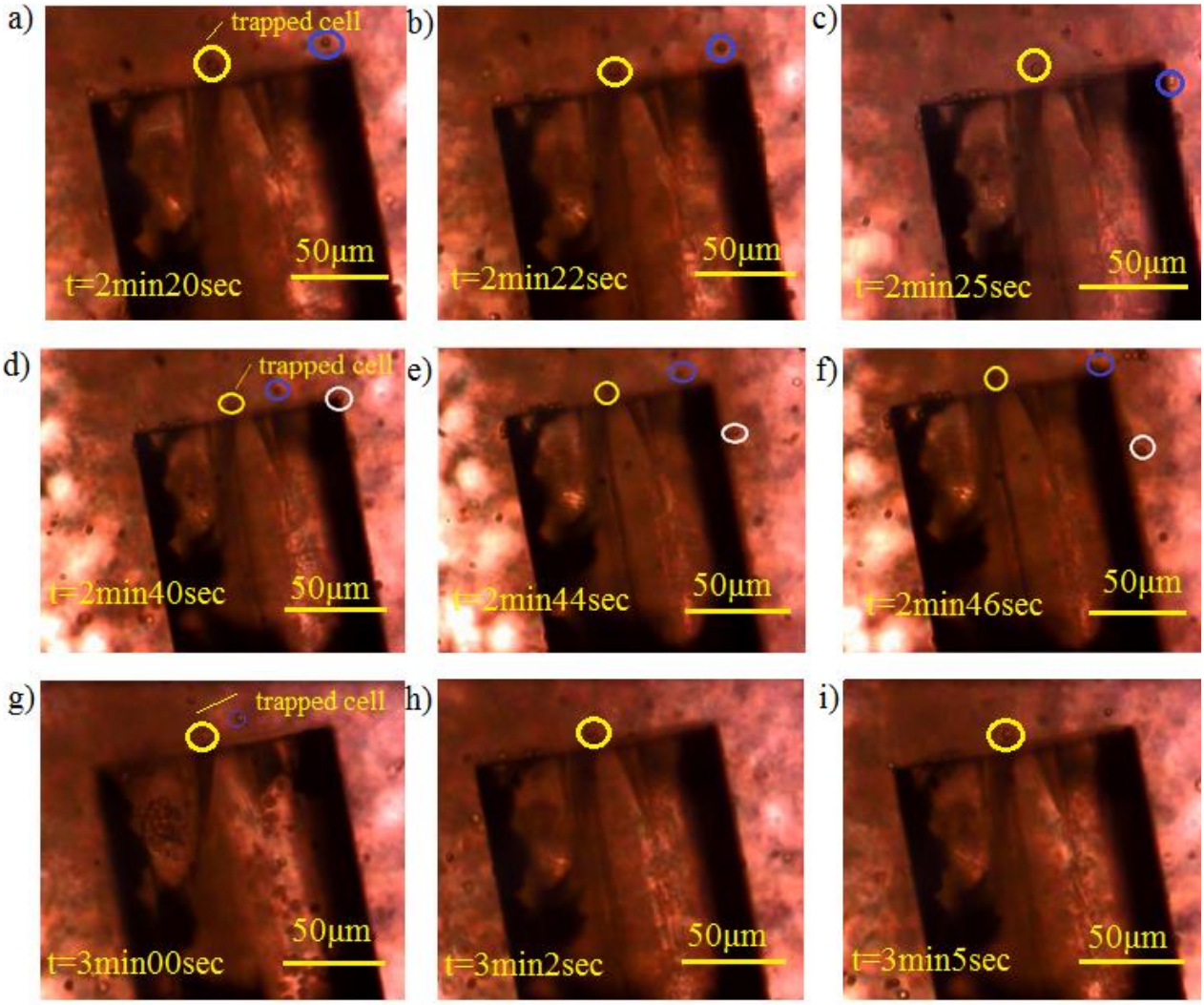

Figure 5: a)-c) x-axis trapping. The particle in the yellow circle is trapped. While the fibre is moving in the x-axis, the particle remains stable in comparison with other particles in black and blue circles that are moving. d)-f) y-axis trapping. The yellow circled trapped particle remains stable during the movement in y direction while the other two particles circled follow a different path. g)-i) z-axis trapping. The trapped particle in yellow circle remains stable during the movement of the probe in z direction.

3.2 Raman microspectroscopy of trapped yeast cells

One benefit of using a single fibre probe is the ability to trap under any microscope. Here, the optical probe was used to trap and manipulate yeast cells for use with a Raman microscope.

The acquisition of Raman spectra from a single cell diluted in a medium has some limitations. The glass that is usually used as a substrate for the sample can be either a borosilicate glass or a quartz glass. Although the quartz glass has a Raman spectrum of lower intensity than borosilicate glass, it does have a spectrum that dominates in intensity, compared to the spectrum of a yeast cell. As seen in Figure 5 (black curve), in the case of a straightforward acquisition of a spectrum from a free single cell on a Quartz glass substrate, the only spectrum that could be seen was noise from the quartz substrate. In contrast, for the same Raman parameters, but for a cell that was optically trapped using our fibre-based probe, a clear Raman spectrum (red curve) that may provide information about the contents of the yeast cell can be acquired.

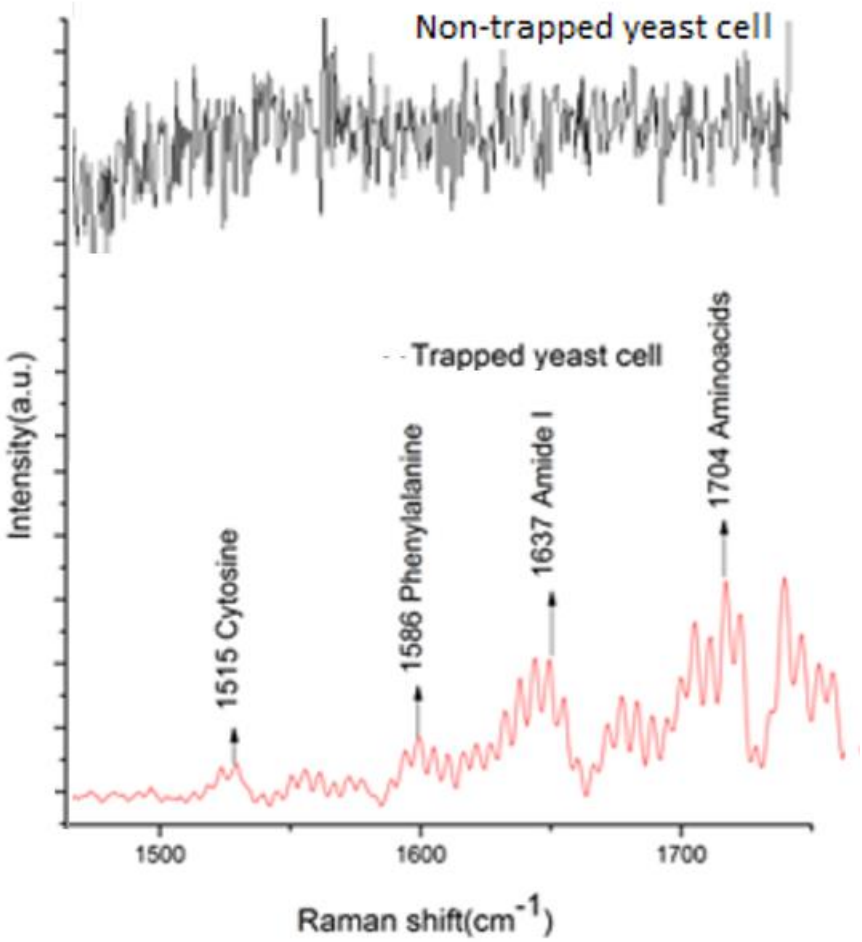

Figure 6: Raman spectra of a free yeast cell in medium and of trapped cell using the fibre-based optical probe.

As a next step, Raman spectra from live yeast cells as well as from dead cells were captured for comparison. We observed differences in the peaks within the Raman spectra that could be attributed to DNA components and proteins, where the live cells produced peaks of stronger signal than the dead cells. These differences could not be observed using light microscopy or by acquiring the Raman spectrum from the cells on a quartz substrate.

4. CONCLUSIONS

In this project, an optical probe based on a machined multicore fibre for optical trapping was demonstrated. Micro-mirrors that can enable the focus of the light close to the fibre end, have been fabricated at two diagonally opposite cores of a multicore fibre using Focused Ion Beam milling. Optical trapping and manipulation of yeast cells of 7μm diameter was demonstrated. Finally, this optical system has been used in combination with a Raman microscope to collect and analyse yeast cells' spectra, decreasing the background spectrum from the sample holder substrate. The machined multicore fibre trap is portable and can be used to trap and manipulate cells under a variety of microscope without relying on a high NA objective to achieve 3D trapping.

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